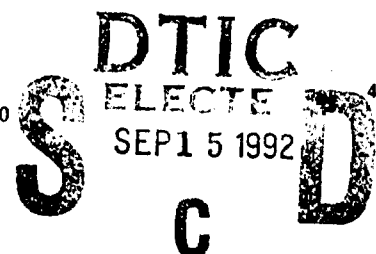




Molecular and Biochemical Parasitology, 53 (1992) 45-52
 © 1992 Elsevier Science Publishers B.V. All rights reserved. 0166-6851/92/\$05.00

MOLBIO 01742



Characterization of the gene encoding sporozoite surface protein 2, a protective *Plasmodium yoelii* sporozoite antigen

William O. Rogers, Miriam D. Rogers, Richard C. Hedstrom* and Stephen L. Hoffman

Malaria Program, Naval Medical Research Institute, Bethesda, MD, USA

(Received 30 December 1991; accepted 6 February 1992)

Sporozoite surface protein 2 (SSP2) is a 140-kDa, protective sporozoite surface protein from *Plasmodium yoelii* distinct from the circumsporozoite protein (CSP). A genomic clone containing the SSP2 gene was isolated and sequenced to determine its size, structural organization and deduced primary amino acid sequence. The coding sequence consists of a single, long open reading frame encoding 826 amino acids. The overall structure of SSP2 is similar to that of the CSP, consisting of a central region of immunogenic amino acid repeats flanked by non-repetitive sequence. SSP2 has one copy of a thrombospondin repeat motif in common with several cell adhesion molecules as well as with the CSP and the thrombospondin related anonymous protein (TRAP) of *P. falciparum*. Additionally, SSP2 shares substantial sequence similarity to TRAP, suggesting that TRAP is the analogue of SSP2 in *P. falciparum*.

Key words: Malaria; *Plasmodium*; Sporozoite; Antigen

Introduction

Efforts to develop a pre-erythrocytic stage malaria vaccine have focused almost entirely on the circumsporozoite protein (CSP)[1]. The CSP is the predominant protein on the surface of the infective malaria sporozoite. It is well known that immunization of humans or animals with radiation attenuated sporozoites induces solid sterile immunity to malaria and both humoral and cellular immune responses

to the CSP [2-6]. Monoclonal antibodies (mAbs) [7,8,10] and cytotoxic T cells [9] directed against the CSP are protective in passive transfer. Nonetheless, it has not been possible to induce active immunity with recombinant or synthetic vaccines based on the CSP alone [8,11-17] comparable to that achieved by immunization with irradiated sporozoites. We have therefore attempted to identify additional sporozoite surface antigens which might be combined with the CSP in a multicomponent vaccine. We recently described a new sporozoite surface antigen, sporozoite surface protein 2 (SSP2), from *Plasmodium yoelii* [5,18]. Monoclonal antibodies directed against SSP2 recognize a 140-kDa protein in sporozoite extracts. Sequence analysis of a 1.5-kb genomic DNA fragment encoding part of SSP2 revealed an immunogenic series of repeating amino acids and a region of similarity to the region II domain of the CSP [1]. Mice immunized with P815 mouse mastocytoma transfectants expressing the partial SSP2 sequence and the CSP were

Correspondence address: William O. Rogers, Malaria Program Naval Medical Research Institute Bethesda, MD 20889-5055, USA. Tel.: (301) 295-1776; Fax: (301) 295-6171.

*Present address: U. S. Naval Medical Research Unit 3, Cairo, Egypt

Note: Nucleotide sequence data reported in this paper have been submitted to the GenBank™ data base with the accession numbers M84732 and M84733.

Abbreviations: CSP, circumsporozoite protein; TRAP, thrombospondin-related anonymous protein; mAb, monoclonal antibody.

92 9 14 021

AD-A256 023
 ORDERED BY: [illegible]
 [illegible]

92-25127



259650

877

1a. REPORT SECURITY CLASSIFICATION UNCL		1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NMRI 92-63		7a. NAME OF MONITORING ORGANIZATION Naval Medical Command	
6a. NAME OF PERFORMING ORGANIZATION Naval Medical Research Institute		6b. OFFICE SYMBOL (If applicable)	
6c. ADDRESS (City, State, and ZIP Code) 8901 Wisconsin Avenue Bethesda, MD 20889-5055		7b. ADDRESS (City, State, and ZIP Code) Department of the Navy Washington, DC 20372-5120	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research & Development Command		8b. OFFICE SYMBOL (If applicable)	
8c. ADDRESS (City, State, and ZIP Code) 8901 Wisconsin Avenue Bethesda, MD 20889-5044		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
		10. SOURCE OF FUNDING NUMBERS	
		PROGRAM ELEMENT NO. 61102A 62770A	PROJECT NO. BM161102BS13 BM162787A870
		TASK NO. AK-111 AN-121	WORK UNIT ACCESSION NO. DA313955 DA317627
11. TITLE (Include Security Classification) Characterization of the gene encoding sporozoite surface protein 2, a protective Plasmodium yoelii sporozoite antigen			
12. PERSONAL AUTHOR(S) Rogers WO, Rogers MD, Hedstrom RC, Hoffman SL			
13a. TYPE OF REPORT journal article		13b. TIME COVERED FROM _____ TO _____	14. DATE OF REPORT (Year, Month, Day) 1992
15. PAGE COUNT 7			
16. SUPPLEMENTARY NOTATION Reprinted from: Molecular and Biochemical Parasitology 1992 Vol. 53 pp. 45-51			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	
19. ABSTRACT (Continue on reverse if necessary and identify by block number)			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL Phyllis Blum, Librarian		22b. TELEPHONE (Include Area Code) (301) 295-2188	22c. OFFICE SYMBOL MRL/NMRI

protected against challenge with infective sporozoites [19]. We report here the characterization of a genomic clone containing the complete SSP2 gene.

Materials and Methods

Parasites and DNA isolation. *P. yoelii* 17X (NL) parasites were maintained and DNA isolation performed as described previously [20].

Genomic library construction and screening. A *P. yoelii* genomic library was constructed using 2.0–7.0-kb fragments generated by partial DNase I digestion as previously described [18]. Purified insert DNA from λ gMSY4 was nick translated and used to screen the library under standard high stringency conditions [21]. Five positive clones were isolated, and one, λ PySSP2.10 was found to contain a 4.7-kb insert which overlapped both ends of λ gMSY4.

DNA sequencing. Phage DNA of λ PySSP2.10 was prepared from liquid lysates by standard methods. Insert DNA was released by *Eco*RI digestion and the inserts were cloned into M13mp18 and pUC18. Overlapping clones spanning the insert were generated in pUC18 and M13 using exonuclease III digestion [22]. Single and double stranded templates were sequenced using the dideoxy method [23] and Sequenase (U.S. Biochemical Corp., Cleveland, OH). Sequence analysis was carried out using Genepro 4.2 and DNASIS software.

Results

A λ gt11 DNase I genomic library was screened with the 1.5-kb fragment of the SSP2 gene contained in λ gMSY4. Five positive clones were obtained. One, λ PySSP2.10, contained a 4.7-kb insert which included within it the complete sequence of the λ gMSY4 sequence. The 4.7-kb insert was subcloned into pUC18 and M13mp18. Nested

deletions were prepared using exonucleaseIII [22] and the complete sequence determined by the Sanger dideoxy method [23].

Fig. 1 shows the sequence of the 4.7-kb insert of λ PySSP2.10. A single long open reading frame is present and includes the previously described sequence of λ gMSY4 [18]. The AT content of the coding and noncoding regions, 63.2% and 80.7% respectively, are similar to those found in other *Plasmodium* genes [24]. The sequence encodes a polypeptide containing 826 amino acids with a calculated molecular weight of 91 300. Several possibilities may account for the discrepancy between this calculated molecular weight and the observed molecular weight, 140 000 [5,18]. First, the gene might contain additional exons. However, no additional long open reading frames were found either 700 bp 5' to the initiation codon or 1500 bp 3' to the first in frame stop codon. No *Plasmodium* consensus intron boundary sequences [24] were found in the flanking regions. A large intron could extend beyond the region we have sequenced, but previously described *Plasmodium* introns have been less than 600 bp long. Second, SSP2 may be a glycoprotein, and indeed, there are several consensus *N*-glycosylation sites in the sequence (Fig. 1). Finally, the protein may migrate anomalously in SDS-PAGE gels, perhaps as a result of its very high proline content.

The deduced amino acid sequence of SSP2 is shown in Fig. 1 and a map of the sequence in Fig. 2. Like a number of other *Plasmodium* surface antigens [24], the deduced amino acid sequence contains tandem repeats of simple amino acid repeats and is particularly rich in proline (18.0%) and asparagine (21.2%). The general structure of SSP2 is similar to that of the CSP (Fig. 2). There is a central region of short, repeated peptide sequences flanked on both sides by non-repetitive sequence. Hydrophobicity analysis [25] identified a putative N-terminal hydrophobic leader [26] as well as putative transmembrane and cytoplasmic domains [27] near the carboxy terminus (Figs. 1 and 2). It is interesting, however, that while SSP2 has both a transmembrane domain and a

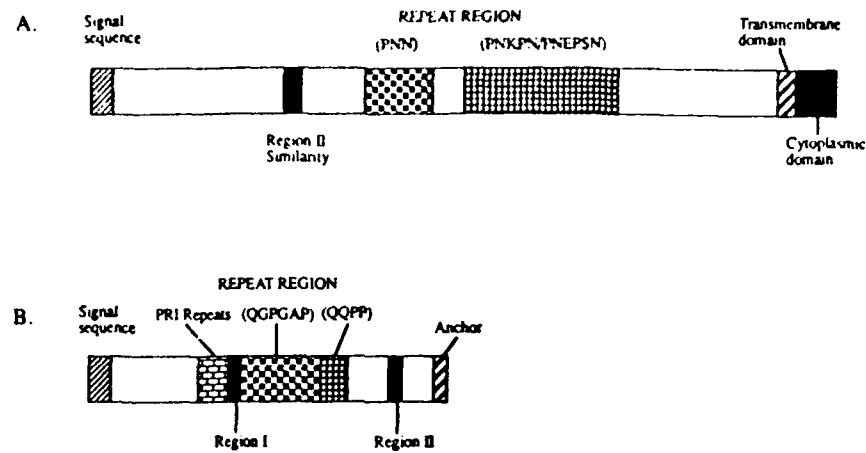


Fig. 2. Structure of SSP2 and CSP. Shown are schematic diagrams of the sequence of SSP2 (A) and the *P. yoelii* CSP (B) [33]. The diagrams are drawn approximately to scale.

is then a short segment of non-repetitive sequence, followed by 3 tandem copies of the pentamer PNKP(K/N), 4 copies of PNKPN

alternating with 4 copies of PNEPSN, and 18 tandem degenerate copies of PNEPSN. These tandem repeats are followed by a short region

A.		
SSP2	MKLLGNSKYIFVVLVLLCISVFL-NGQE----TLDEIKYSEEVCTEQIDIHILLDGGSGSIG	55
TRAP	MNHLGNVVKYLVIVFLIFFDLFLVNGRDVQNNIVDEIKYSEEVCTEQVDLYLLMDCSGSIR	60
SSP2	YSNWKAHVIPMLNTLVNDNLNISNDEINVSLLTFSNLSRELKIKGYGSTSKDLSRFLAH	115
TRAP	RHNWVNHAVPLAMKLIQQLNLNDNATHLYVNVFSNNAKEIIRLHSDASKNEKALIIIRS	120
SSP2	LQNNYSPNGNTNLTSAALLVVDTLINERMYRPDAIQLAIIITDGPNDLPRSTAVVHQLKR	175
TRAP	LLSTNLPYGRNTLTDALLQVRKHLNDRINRENANQLVVILTDPDSIQDSLKESRKLSD	180
SSP2	KHVNVAIIGVGAGVNNENRILVGCDDRY-APCPYSSGSWNEAQNMIKPFLTKVCQEVER	234
TRAP	RGVKIAVFGIGQGINVAFNRFLVGCHPSDGKCNLYADSAWENVKNVIGPFMKAVCVEVEK	240
SSP2	IAHCGKWEEN <u>SECSTTC</u> DEGRKIRRRQILHPGCVSEMTTPCKVRDCP	281
TRAP	TASCGVWDE <u>NSPCS</u> VTCGKGRSRKREILHEGCTSEIQEQCEEERCP	287
B.		
SSP2	SNNGYKIAGGIIGGLAILGCAGVGYNFIAGSSAAGLAGAEPAPFEDVIPDDDKDIVENEQ	816
TRAP	SDNKYKIAGGIAGGLALLACAGLAYKFVVPGAATPYAG-EPAPFDETLGEEDKDLDEPEQ	550
SSP2	FKLPEDNDMN	826
TRAP	FRLPEENEMN	560

Fig. 3. Alignment of the N-terminal (A) and C-terminal (B) regions of *P. yoelii* SSP2 and *P. falciparum* TRAP. ':' indicates identical amino acids, '.' indicates conservative substitutions. The thrombospondin repeat motif is underlined.

in which short repeat motifs, PEE and PSN, are interspersed with non-repetitive sequence. Finally, there is a tandem duplication of the 11-mer, PEESNPKEPIN. All of the repeat sequences in SSP2 are clearly distinct from the repeats in the *P. yoelii* CSP, QGPGAP and QQPP. The only common feature which the SSP2 repeats share with the CSP repeats is the general structure PXXPXX, which might be expected to impart to the repeat domains of both proteins a structure rich in β -bends [28].

SSP2 shares sequence motifs with several plasmodial proteins and molecules involved in cell adhesion. Thrombospondin, the CSP region II, properdin, the terminal complement components and the thrombospondin related anonymous protein share similarities based around the nonapeptide, WSPCSVTCG [29,30]. This sequence is found in 3 copies in thrombospondin, 6 copies in properdin and one copy in all CS proteins sequenced to date. A similar sequence, underlined in Fig. 1, is also found in SSP2. In SSP2 this thrombospondin motif is found amino terminal to the central repeat region, while the analogous sequence in the CSP is found in Region II, carboxy terminal to the repeats.

The N-terminal and C-terminal regions of SSP2 bear a remarkable similarity to TRAP which extends well beyond the similarity to the thrombospondin motif. Fig. 3A shows an alignment of the N-terminal regions of SSP2 and TRAP. Over a region of 281 amino acids, there is 43% similarity at the amino acid level. Ten of 11 cysteine residues are conserved, the only exception being a single cysteine in the putative hydrophobic leader of SSP2. Fig. 3B show the alignment of the C-terminal regions of the 2 proteins. Over a region of 71 amino acids, there is 56% identity at the amino acid level. SSP2 and TRAP may be members of a protein family involved in interaction between the sporozoite and erythrocytic stages of *Plasmodium* and the cells of the host.

Discussion

SSP2 is a new, non-CSP, 140-kDa sporo-

zoite surface antigen from *P. yoelii* [18]. We have recently observed that immunization of mice with a combination of P815 mouse mastocytoma transfectants expressing the CSP and the original 1.5-kb fragment of SSP2 [18] are protected against challenge with infective *P. yoelii* sporozoites [19]. SSP2 and its presumed homologs in the human *Plasmodium* species are therefore important vaccine candidates. We have here reported the complete sequence of the *P. yoelii* SSP2 gene.

The deduced amino acid sequence of SSP2 shares a number of characteristics with other *Plasmodium* surface antigens in general and with the CS protein in particular. First, it is characterized by a central repeat region consisting of tandem repeats of several different short peptide sequences. As in the case of the CSP repeats from many *Plasmodium* species, the amino acids used in the SSP2 repeats are chosen from a restricted set of amino acids (P,N,E,Q,G,D,A,R,V for CSP repeats and P,N,E,K,S,I for the SSP2 repeats). As in the *P. yoelii* CSP, there are several different repeat units in the repeat region, however, the organization of the repeats in SSP2 is somewhat more complex than that in the CSP. There are 2 major repeat regions, one consisting of tandem repeats of the tripeptide PNN, the second consisting of 2 basic repeat units, PNKPN and PNEPSN. The units are intercalated in the general structure AAAABABABABBBB, where A = PNKPN and B = PNEPSN. This organization could have arisen from an ancestral 11-mer repeat unit AB = PNKPNPNEPSN by duplication of the component 5- and 6-mers at the amino and carboxy terminal ends of an ancestral 11-mer repeat region. One would expect that over the course of evolutionary time the central, alternating AB repeats could be eliminated by homologous recombination, resulting in a simpler AAAAABBBBBB organization, which is in fact observed in a number of CSP repeat regions [31-33]. The SSP2 repeat region may, therefore, represent an intermediate step in a general mechanism in *Plasmodium* antigen genes by which an ancestral tandem duplication of a relatively long sequence evolves into a

repeat region characterized by tandem repeats of 2 or more different short peptide sequences.

Sporozoites which have been inoculated into the mammalian host progress rapidly from the circulation to infect hepatocytes. It is likely that rapid homing to the liver requires specific cell-cell interaction between the sporozoite and hepatocytes, Kupffer cells, or endothelial cells in the hepatic circulation. It is thus interesting to find that SSP2 shares a sequence with the cell adhesion molecules, thrombospondin, properdin, and the terminal complement components, as well as with the CSP Region II and another *Plasmodium* antigen, thrombospondin related anonymous protein (TRAP). These thrombospondin motifs are centered on the nonapeptide WSPCSVTCG, and are found in 3 copies in thrombospondin, 6 copies in properdin, and one copy in all CS proteins sequenced to date [29,30]. SSP2 may also have a role in cell-cell interactions between the sporozoite and the mammalian host.

SSP2 bears a striking similarity to TRAP which extends considerably beyond the thrombospondin repeat motif. The first 281 amino acids of SSP2 and TRAP have a 43% similarity at the amino acid level. Ten of 11 cysteines in the amino terminal sequence of SSP2 are identically conserved in TRAP, the only exception being a single cysteine in the putative hydrophobic leader of SSP2. A region of 56% identity extending over 71 amino acids is found at the carboxy terminus. The similarity in overall structure, as well as the striking amino acid sequence similarities at the amino and carboxy termini strongly suggest that TRAP is the *P. falciparum* analogue of SSP2. It is interesting that has a large repeat region, while TRAP, an apparently closely related protein, has none. If SSP2 and TRAP are indeed analogous proteins with the same function, the absence of repeats in TRAP may call into question the functional importance of repeats in *Plasmodium* antigens generally.

Acknowledgements

We thank Stephen Merritt for helpful discussions. This research was supported by

the Naval Medical Research and Development Command Project No. 3M16102BS13AK111 and 3M162787A870AN121. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

References

- 1 Nussenzweig, V. and Nussenzweig, R. S. (1985) Circumsporozoite proteins of malaria parasites. *Cell* 42, 401-403.
- 2 Nussenzweig, R. S., Vanderberg, J. P., Most, H. and Orton, C. (1969) Protective immunity induced by the injection of X-irradiated sporozoites of *Plasmodium berghei*. *Nature* 216, 160-162.
- 3 Clyde, D. F., McCarthy, V. C., Miller, R. M. and Woodward, W. E. (1975) Immunization of man against falciparum and vivax malaria by use of attenuated sporozoites. *Am. J. Trop. Med. Hyg.* 24, 397-401.
- 4 Rieckman, K. H., Carson, P. E., Beaudoin, R. L., Casells, J. S. and Sell, K. W. (1974) Sporozoite-induced immunity in man against an Ethiopian strain of *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg.* 68, 258-259.
- 5 Charoenvit, Y., Leef, M. F., Yuan, L. F., Sedegah, M., and Beaudoin, R. L. (1987) Characterization of *Plasmodium yoelii* monoclonal antibodies against stage specific sporozoite antigens. *Infect. Immun.* 55, 604-608.
- 6 Weiss, W. R., Mellouk, S., Houghten, R. A., Sedegah, M., Kumar, S., Good, M. F., Berzofsky, J. A., Miller, L. H. and Hoffman, S. L. (1990) Cytotoxic T cells recognize a peptide from the circumsporozoite protein on malaria infected hepatocytes. *J. Exp. Med.* 171, 763-773.
- 7 Potocnjak, P., Yoshida, N., Nussenzweig, R. S. and Nussenzweig, V. (1980) Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection. *J. Exp. Med.* 151, 1504-1513.
- 8 Charoenvit, Y., Mellouk, S., Cole, C., Bechara, R., Leef, M. F., Sedegah, M., Yuan, L. F., Robey, F. A., Beaudoin, R. L. and Hoffman, S. L. (1991) Monoclonal, but not polyclonal, antibodies protect against *Plasmodium yoelii* sporozoites. *J. Immunol.* 146, 1020-1025.
- 9 Romero, P., Maryanski, J. L., Corradin, G., Nussenzweig, R. S., Nussenzweig, V. and Zavala, F. (1989) Cloned cytotoxic T cells recognize an epitope in the circumsporozoite protein and protect against malaria. *Nature* 341, 323-326.
- 10 Charoenvit, Y., Collins, W. E., Jones, T. R., Millet, P., Yuan, L., Campbell, G. H., Beaudoin, R. L., Broderick, J. R. and Hoffman, S. L. (1991) Inability of a malaria vaccine to induce antibodies to a protective epitope within its sequence. *Science* 251, 668-671.
- 11 Ballou, W. R., Hoffman, S. L., Sherwood, J. A., Hollingdale, M. R., Neva, F. A., Hockmeyer, W. T., Gordon, D. M., Schneider, I., Wirtz, R. A., Young, J. F., Wasserman, G. F., Reeve, P., Diggs, C. L., and Chulay, J. D. (1987) Safety and efficacy of a

- recombinant DNA *Plasmodium falciparum* sporozoite vaccine. *Lancet* i, 1277-1281.
- 12 Herrington, D. A., Clyde, D. F., Losonski, G., Cortesia, M., Murphy, J. R., Davis, J., Baqar, S., Felix, A. M., Heimier, E. P., Gillespie, D., Nardin, E., Nussenzweig, R. S., Nussenzweig, V., Hollingdale, M. R. and Levine, M. M. (1987) Safety and immunogenicity of a synthetic peptide malaria vaccine against *Plasmodium falciparum* sporozoites. *Nature* 328, 257-259.
 - 13 Collins, W. E., Nussenzweig, R. S., Ballou, W. R., Ruebush, T. K., Nardin, E. H., Chulay, J. D., Marjarian, W. R., Young, J. F., Wasserman, G. F., Broderick, J. R., Skinner, J. C., Procell, P. M., Filipski, V. K. and Wilson, C. L. (1989) Immunization of *Saimiri sciureus boliviensis* with recombinant vaccines based on the circumsporozoite protein of *Plasmodium vivax*. *J. Trop. Med. Hyg.* 40, 455-464.
 - 14 Egan, J. E., Weber, J. L., Ballou, W. R., Hollingdale, M. R., Marjarian, W. R., Gordon, D. M., Maloy, W. L., Hoffman, S. L., Wirtz, R. A., Schneider, I., Woollett, G. R., Young, J. F. and Hockmeyer, W. T. (1987) Efficacy of murine malaria sporozoite vaccines: implications for human vaccine development. *Science* 236, 453-456.
 - 15 Zavala, F., Tam, J. P., Barr, P. J., Romero, P. J., Ley, V., Nussenzweig, R. S., and Nussenzweig, V. (1987) Synthetic peptide vaccine confers protection against murine malaria. *J. Exp. Med.* 166, 1591-1596.
 - 16 Sedegah, M., Beaudoin, R. L., Marjarian, W. R., Chiang, C., Cochran, M. D., Sadoff, J., Aggarwal, A., Charoenvit, Y. and Hoffman, S. L. (1990) Evaluation of vaccines designed to induce protective cellular immunity against the *Plasmodium yoelii* circumsporozoite protein: vaccinia, pseudorabies, and salmonella transformed with circumsporozoite gene. *H. Bull. World Health Org.* 68 (Suppl.), 109-114.
 - 17 Sadoff, J. C., Ballou, W. R., Baron, L. S., Marjarian, W. R., Brey, R. N., Hockmeyer, W. T., Young, J. F., Cryz, S. J., Ou, J., Lowell, G. H., Chulay, J. D. (1988) Oral Salmonella typhimurium vaccine expressing circumsporozoite protein protects against malaria. *Science* 240, 336-338.
 - 18 Hedstrom, R. C., Campbell, J. R., Leef, M. L., Charoenvit, Y., Carter, M., Sedegah, M., Beaudoin, R. L. and Hoffman, S. L. (1990) A malaria sporozoite surface antigen distinct from the circumsporozoite protein. *Bull. World Health Org.* 68 (Suppl.), 152-157.
 - 19 Khusmith, S., Charoenvit, Y., Kumar, S., Sedegah, M., Beaudoin, R. L. and Hoffman, S. L. (1991) Protection against malaria by vaccination with sporozoite surface protein 2 plus CS protein. *Science* 252, 715-718.
 - 20 Wortman, A., Rogers, P., Charoenvit, Y., McDermott, A., Leef, M., Sedegah, M. and Beaudoin, R. L. (1989) Cloning of *Plasmodium yoelii* genes expressing three different sporozoite specific antigens. *Micro. Path.* 6, 227-231.
 - 21 Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1987) *Current Protocols in Molecular Biology*. Greene Wiley Interscience, New York.
 - 22 Henikoff, S. (1984) Unidirectional digestion with Exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* 28, 351-359.
 - 23 Sanger, F., Nicklen, S. and Coulson, A. R. (1977) DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74, 5463-5467.
 - 24 Weber, J. L. (1988) Molecular biology of malaria parasites. *Exp. Parasitol.* 66, 143-170.
 - 25 Kyte, J. and Doolittle, R. F. (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 157, 105-132.
 - 26 von Heijne, G. (1985) Signal sequences: The limits of variation. *J. Mol. Biol.* 184, 99-105.
 - 27 Jennings, M. L. (1989) Topography of membrane proteins. *Annu. Rev. Biochem.* 58, 999-1027.
 - 28 Chothia, C. (1984) Principles that determine the structure of proteins. *Annu. Rev. Biochem.* 53, 537-572.
 - 29 Robson, K. J. H., Hall, J. R. S., Jennings, M. W., Harris, T. J. R., Marsh, K., Newbold, C. I., Tate, V. E. and Weatherall, D. J. (1988) A highly conserved amino-acid sequence in thrombospondin, properdin and in proteins from sporozoites and blood stages of a human malaria parasite. *Nature* 335, 79-82.
 - 30 Goundis, D. and Reid, K. B. M. (1988) Properdin, the terminal complement components, thrombospondin and the circumsporozoite protein of malaria parasites contain similar sequence motifs. *Nature* 335, 82-85.
 - 31 Weber, J. L., Egan, J. E., Lyon, J. A., Wirtz, R. A., Charoenvit, Y., Maloy, W. L. and Hockmeyer, W. T. (1987) *Plasmodium berghei*: cloning of the circumsporozoite protein gene. *Exp. Parasitol.* 63, 295-300.
 - 32 Galinski, M. R., Arnot, D. E., Cochrane, A. H., Barnwell, J. W., Nussenzweig, R. S. and Enea, V. (1987) The circumsporozoite gene of the *Plasmodium cynomolgi* complex. *Cell* 48, 311-319.
 - 33 de la Cruz, V. F., Lal, A. A. and McCutchan, T. F. (1988) Variation among circumsporozoite protein genes from rodent malarias. *Mol. Biochem. Parasitol.* 28, 31-38.

DTIC QUALITY INSPECTED 3

Accession For	
DTIC	<input checked="" type="checkbox"/>
DTIC File	<input type="checkbox"/>
Microfilm	<input type="checkbox"/>
Full Text	<input type="checkbox"/>
3	
Availability Codes	
Unlimited and/or	
Special	
A-1	20